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EXOGENOUS METHYL JASMONATE INDUCES VOLATILE EMISSIONS IN COTTON PLANTS

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Abstract—We investigated the effect of exogenous methyl jasmonate (MeJA) on the emission of herbivore-induced volatiles; these volatile chemicals can signal natural enemies of the herbivore to the damaged plant. Exogenous treatment of cotton cv. Deltapine 5415 plants with MeJA induced the emission of the same volatile compounds as observed for herbivore-damaged plants. Cotton plants treated with MeJA emitted elevated levels of the terpenes (*E*)-β-ocimene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*,*E*)-α-farnesene, (*E*)-β-farnesene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene compared to untreated controls. Other induced components included (*Z*)-3-hexenyl acetate, methyl salicylate, and indole. Methyl jasmonate treatment did not cause the release of any of the stored terpenes such as α-pinene, β-pinene, α-humulene, and (*E*)-β-caryophyllene. In contrast, these compounds were emitted in relatively large amounts from cotton due to physical disruption of glands by the herbivores. The timing of volatile release from plants treated with MeJA or herbivores followed a diurnal pattern, with maximal volatile release during the middle of the photoperiod. Similar to herbivore-treated plants, MeJA treatment led to the systemic induction of (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*,*E*)-α-farnesene, (*E*)-β-farnesene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Our results indicate that treatment of cotton with MeJA can directly and systemically induce the emission of volatiles that may serve as odor cues in the host-search behavior of natural enemies.

Key Words—Cotton, *Spodoptera exigua*, methyl jasmonate, induction, plant defense, volatile semiochemicals.

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INTRODUCTION

Plants protect themselves against herbivory by deploying a wide array of chemical defenses (Karban and Myers, 1989; Agrawal et al., 1999). Wounding-induced plant responses can directly target herbivores by stimulating the synthesis of toxic or antifeedant metabolites, as well as by activating antinutrient enzymes such as proteinase inhibitors or polyphenol oxidases (e.g., Felton et al., 1994; Stout et al., 1996). Plant volatiles can also serve as a chemical defense by recruiting beneficial insects that are natural enemies of the herbivore, thereby providing an indirect protection to the plant (Dicke and Vet, 1999; Paré and Tumlinson, 1999). These volatile organic compounds increase the foraging success of natural enemies by providing reliable odor cues for prey/host location (Dicke and Sabelis, 1988; Dicke et al., 1990; Turlings et al., 1995).

Currently, there is increasing evidence of a common biosynthetic pathway linking direct and indirect induced plant defenses. Wounding of leaf tissue activates the octadecanoid/lipoxygenase (LOX) pathway, a lipid-based signaling sequence, resulting in the accumulation of 12-oxyphytodienoic acid and 7-isojasmonic acid (JA) (Farmer and Ryan, 1990; Constabel and Ryan, 1998). The involvement of JA in regulating gene activation subsequent to wounding has been established in several plant species (Wasternack et al., 1998; Staswick and Lehman, 1999). The LOX pathway also plays an important role in regulation of indirect plant defenses. Volatile emissions can be stimulated by exogenous JA (see Boland et al., 1998, for review) and by a select group of amino acid conjugates of JA (Krumm et al., 1995) taken up through the stems of a plant. Another connection of the LOX signaling pathway with volatile synthesis and emissions was the identification of *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), an herbivore-specific elicitor that is derived from linolenic acid and induces volatile production (Alborn et al., 1997). Incubation of amino acid conjugates of linolenic acid (e.g., linolenoyl-L-glutamine) mimics the action of 12-oxophytodienoic acid (PDA), an early intermediate on the LOX pathway, in inducing emission of a blend of volatiles in a Lima bean cut seedling bioassay (Koch et al., 1999). This elicitor response appears to be highly specific since *N*-(17-hydroxylinoleoyl)-L-glutamine, a compound closely related to volicitin, is ineffective in inducing volatile emissions (Alborn et al., 2000).

In several agricultural species that have been studied, volatile chemicals emitted from both damaged and undamaged portions of herbivore-injured leaves serve as essential host-location cues for parasitic insects (see review by Dicke and Vet, 1999). Chemical labeling studies have established that a subset of volatiles emitted from cotton (*Gossypium hirsutum* L.) damaged by insects is synthesized and rapidly emitted from damaged and undamaged leaves, while other chemical constituents, stored in specialized glands, are volatilized only from the leaves that are damaged by insects (Paré and Tumlinson, 1997a,b, 1998). In mint leaves, such

glands contain enzymes required for terpene synthesis (Gershenzon et al., 1989). By directly comparing volatile emission patterns of glanded cotton plants treated with jasmonates or damaged by herbivores, compounds induced by jasmonates can be distinguished from those that are emitted due to mechanical damage.

Our objectives were to compare the emission of volatiles from cotton plants treated with methyl jasmonate (MeJA), a volatile derivative of JA, or subjected to herbivore damage. Specifically, we determined the diurnal pattern of volatile emission induced by these treatments. Finally, we report that MeJA induces the systemic release of the same volatile compounds that are systemically induced by herbivore feeding.

METHODS AND MATERIALS

Plants and Insects

Four- to 6-week-old glanded cotton, *G. hirsutum* cv. Deltapine 5415, plants with four to six fully expanded true leaves were used in all experiments. Cotton was grown in air-conditioned greenhouses under natural light (2000 μmol/m^2/sec^{-1} max daily photosynthetically active radiation) and Arizona fall–spring conditions (11L:13D photoperiod, and 28°C day, 24°C night). Plants were grown in 15×15-cm pots containing a commercial potting mixture (Grow More, Gardena, California) and fertilized three times a week with 750 ml of a solution containing 2 g/liter Grow More 20-20-20 fertilizer. The nutrient solution was supplemented with 0.5 ml/l micronutrient solution containing 2 mM $MnCl_2$, 10 mM H_3BO_3, 0.4 mM $ZnSO_4$, 0.2 mM $CuSO_4$, 0.4 mM Na_2MoO_4, and 0.1 mM $NiCl_2$. All plants employed were free of insects and mites.

The generalist beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), was used in all experiments because cotton plants damaged by this herbivore emit a blend of volatiles important for parasitic insects in host location (Loughrin et al., 1994; Röse et al., 1996). Larvae were reared on an alfalfa-based diet (Henneberry and Kishaba, 1966) at 28°C and 50% relative humidity. The colony has been maintained in culture at the Western Cotton Research Lab., USDA-ARS, Phoenix, Arizona for more than 10 years, and had new genetic material added within 12 months prior to the study from the Southern Insect Management Research Unit, USDA-ARS, Stoneville, Mississippi.

Volatile Collection System

Volatile chemicals emitted by individual cotton plants were collected in a push/pull apparatus constructed after Heath and Manukian (1994). The system consisted of four independent glass chambers that allowed for simultaneous collections. Air entering each of the glass chambers initially was split into two

directions: in one, air was pushed through a flowmeter (to measure and regulate the amount of air) followed by a charcoal filter (to purify the air) and a humidifier bubbler (wet air). Air in the second direction was pushed through a flowmeter and a charcoal filter (dry air). Both moist and dry air entered a glass cylinder (42.5 cm high and 18 cm diameter; Analytical Research Systems, Inc., Gainesville, Florida) at 5 L/min (Loughrin et al., 1994; Röse et al., 1996). The air passed over a cotton plant placed inside the cylinder through the opened bottom (see Röse et al., 1996; Turlings et al., 1998, for details). The bottom of the chamber was then sealed by using a guillotine-like support base (Analytical Research Systems, Inc.) leaving a hole in the center around the stem of the plant. The system allows sampling volatiles from the aerial parts (all of its leaves) of a plant, while the pot remains outside. At 5 cm from the base of the cylinder, eight openings allowed for attachment of collection filter traps (Analytical Research Systems, Inc.). Collection traps consisted of 10 cm long and 6 mm diameter glass filters containing 30 mg of Super-Q adsorbent (Alltech Assoc. Inc., Deerfield, Illinois). Manual switching valves allowed the sample to be diverted to individual filter traps, thus facilitating volatile collection at various times of the day.

The tip of each collection trap was placed a few millimeters from the plant. During collection, air was pulled through one of the collection traps at a rate of 1 l/min (Loughrin et al., 1994; Röse et al., 1996). Thus, only 20% of the air passed through the collector trap, the remaining escaped through the opening at the bottom of the guillotine base, which was loosely closed with cotton to prevent abrasion (see Röse et al., 1996; Turlings et al., 1998).

Analysis of Headspace Volatiles

Volatiles were extracted from the collector traps by rinsing them with 180 μl of methylene chloride. An internal standard (600 ng of *n*-octane in 5 μl of methylene chloride) was added to the extract. From each sample, 1 μl was analyzed on a Hewlett-Packard model 6890 gas chromatograph (GC) equipped with a capillary injector system and flame ionization detector. Samples were injected by a Hewlett-Packard auto injector model 7683, programmed in a split mode (25:1). All samples were analyzed on a HP1 methyl siloxane column (30 m $\times$ 0.32 mm ID, 0.25 μm film). Helium at a linear flow velocity of 40 cm/sec was used as a carrier gas. Following injection, column temperature was maintained at 50°C for 3 min and then increased at 5°C/min to 190°C and maintained at 190°C for 5 min. Data were analyzed with Hewlett-Packard ChemStation software. For each sample, amounts of the detected volatiles were based on comparison of their peak areas with that of the internal standard.

Selected samples were analyzed by GC–mass spectroscopy (GC-MS) with a Hewlett-Packard model 5973 mass selective detector operated at an initial temperature of 40°C for 1 min, then programmed at 14°C/min to 180°C. The column

oven was maintained at 180°C for 2 min and then raised at 40°C/min to 200°C and held for 2 min. For GC-MS samples, a DB5-MS column (J&W Scientific, Folsom, California) 30 m × 0.25 mm ID, with a 0.1-μm-thick film of bonded methyl silicone with 5% phenyl, was used. Spectral data were compared with commercially available standards and spectra from the National Institute of Standards and Technology (NIST, 1995) database.

Comparison of Cotton Volatiles Induced by Herbivory versus MeJA Treatment

Volatiles were collected from cotton damaged by *S. exigua* larvae, treated with MeJA, and controls. Volatiles were collected as previously described beginning at 10:00 hr for a total of 22 hr. At least one control was run simultaneously with other treatments at a particular collection time. Treatments were replicated five times.

Herbivore-Damaged Plants. A cohort of recently molted third instar *S. exigua* from the colony was transferred from artificial diet cups to cotton plants (approx. 10 larvae/plant) 12 hr prior to the experiment to allow the larvae to habituate to the new diet. Larvae were allowed to feed on cotton overnight. The following morning larvae were removed from the plants, starved for 2 hr to encourage immediate feeding, and placed inside a collection chamber with a new cotton plant. At 10:00 hr, 10 larvae were placed inside a volatile collection chamber containing a plant and allowed to feed *ad libitum* over 22 hr.

Methyl Jasmonate-Treated Plants. Cotton was treated with MeJA (Aldrich, Milwaukee, Wisconsin) overnight, starting at 16:00 hr, in the greenhouse. Plants were treated by applying 20 μl of an ethanol–MeJA (9:1) solution (after Thaler et al., 1996; Constabel and Ryan, 1998) onto a 15-cm cotton tipped applicator (Fisher, Pittsburgh, Pennsylvania). Two treated cotton wicks (total of 18 μmol MeJA/plant) were placed underneath cotton leaves, without physically contacting the treated plants. Plants were treated inside a Plexiglas cylindrical chamber (26 cm diameter × 60 cm high with the top of the chamber and a 14-cm-diameter opening in the middle covered by a fine nylon mesh to allow air circulation). Control cotton plants were exposed to 40 μl ethanol but no MeJA and placed in separate chambers under the same conditions. Plants were kept in the chambers for 18 hr, after which cotton wicks from the treated plants were removed and plants were placed inside the chambers for volatile collection.

Timing of Induction

Volatiles released over time were collected from cotton damaged by *S. exigua* larvae, treated with exogenous MeJA, and controls. At least one control was run simultaneously with other treatments at a particular collection time. Volatiles were collected for 4-hr periods over two consecutive days: on day 1 at 10:00, 14:00, 18:00, and 22:00 hr; and on day 2 at 06:00, 10:00, 14:00, and 18:00 hr. Treatments

were replicated four times. In addition, daily fluctuation in leaf temperature was monitored for plants inside the collection chamber by using a thermocouple pressed to the bottom of a fully expanded leaf.

Herbivore-Damaged Plants. In the first series of experiments 10 *S. exigua* larvae were allowed to feed on cotton plants overnight, starting at 16:00 hr. Larvae were kept on the leaves by enclosing them inside a fine-mesh nylon bag sealed around the leaf petiole with Velcro. The following day (06:00 hr) larvae and plants were placed inside collection chambers (see above) for volatile collections. In these experiments, caterpillars were allowed to feed continuously. In a second set of experiments, larvae were also allowed to feed on cotton plants overnight, starting at 16:00 hr; however, herbivore feeding was stopped by removing larvae from the plants at the time when the plants were placed inside the collection chambers at 06:00 hr.

Methyl Jasmonate-Treated Plants. Starting at 16:00 hr, plants were placed inside Plexiglas cylinders and treated with MeJA as previously described. The following morning at 06:00 hr, cotton wicks were removed and plants were placed inside collection chambers and volatiles were collected. In addition, control plants were placed under the same conditions but with no exposure to MeJA.

Systemic Induction

In order to maximize treatment differences, systemically induced volatiles were collected in the same filter trap during the photoperiod for four consecutive days. Volatile collection was started at 10:00 hr each day and ended at 20:00 hr on days 1 to 3, and at 18:00 hr on day 4. At least one control was run simultaneously with other treatments at a particular collection time. Treatments were replicated 4 times.

Herbivore-Damaged Plants. Four beet armyworm larvae were enclosed in a fine-mesh nylon bag and allowed to feed on the lower two leaves of a cotton plant, while volatiles were collected from the undamaged upper leaves. Larvae started feeding on the lower leaves 18 hr before the beginning of the volatile collections.

Methyl Jasmonate-Treated Plants. Systemic response of cotton treated with MeJA was tested by treating the two lower leaves on a plant with 20 μl of an ethanol– MeJA (9:1) solution (total of 18 μmol MeJA/plant). Leaves and the cotton wicks treated with MeJA were enclosed in a perforated Ziploc plastic bag. Lower leaves were treated with MeJA at 16:00 hr the day prior to volatile collections and were treated with MeJA daily at 16:00 hr by reapplying 18 μmol of MeJA to the cotton wick. For controls, the lower two leaves were enclosed in plastic bags containing cotton wicks treated with 20 μl of ethanol each, but received no MeJA treatment.

Statistical Analyses

A completely randomized one-way ANOVA (SigmaStat, San Rafael, California) was used to determine differences in total volatile emissions among treatments (herbivore, MeJA, and controls). If significant differences were detected, an all pairwise multiple comparison procedure (Tukey test) was conducted. Differences in individual volatile components among treatments were determined by using the MULTTEST PROC (SAS Institute Inc., Cary, North Carolina).

RESULTS

Comparison of Cotton Volatiles Induced by Herbivory versus MeJA Treatment. Differences in volatile emissions were observed for cotton plants damaged by *S. exigua* larvae, treated with MeJA, or undamaged controls (Table 1). Control plants emitted much lower amounts of volatiles compared to herbivore damaged plants and plants treated with MeJA (Table 1).

Plants treated with herbivores or MeJA emitted a blend that consisted of (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, indole, (*E*,*E*)-α-farnesene, (*E*)-β-farnesene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Figure 1). These compounds are commonly referred to as inducible because they are synthesized *de novo* by herbivore-damaged cotton (Paré and Tumlinson, 1997a,b). Other detected components were methyl salicylate and an unidentified sesquiterpene (Figure 1). Control plants also emitted the inducible linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Figure 1; see also Röse et al., 1996).

The terpenes α-pinene, β-pinene, myrcene, limonene, (*E*)-β-caryophyllene, and α-humulene were detected only from herbivore-damaged cotton (MULTTEST;

TABLE 1. TOTAL AMOUNT OF VOLATILES EMITTED LOCALLY AND SYSTEMICALLY FROM COTTON PLANTS DAMAGED BY *S. exigua* LARVAE TREATED EXOGENOUSLY WITH METHYL JASMONATE AND CONTROL PLANTS

	Amount	
Treatment	Local[a] (ng/hr ± SE)	Systemic[b] (ng ± SE)
S. exigua damage	649.9 ± 153.3 a[c]	10317.2 ± 3005.8 a
Methyl jasmonate	573.4 ± 147.8 a	5173.6 ± 1388.3 a
Control	139.5 ± 50.2 b	376.3 ± 121.8 b

[a] Volatiles collected continuously for 22 hr; $N = 5$.
[b] Cumulative volatiles emitted during the Photoperiod of 4 consecutive days were collected; $N = 4$.
[c] Different letters within columns indicate statistical differences among treatments (Local: $F = 6.4; df = 2,14; P = 0.013$; systemic: $F = 6.8; df = 2,11; P = 0.016$; Tukey test; $P < 0.05$).

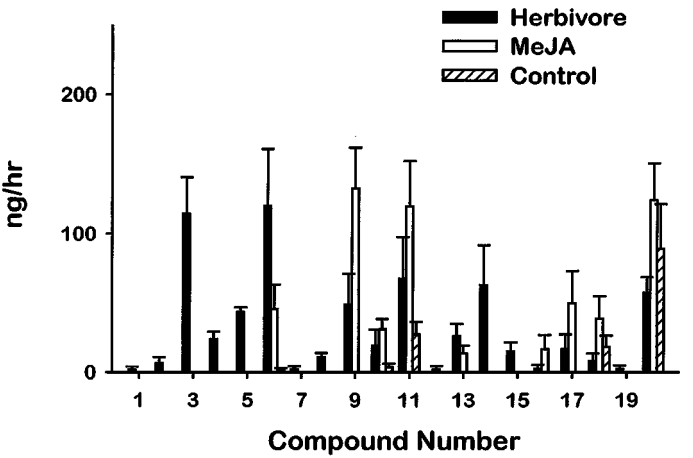

FIG. 1. Volatiles collected from the aerial portions of undamaged cotton plants (Control), plants damaged by *S. exigua*, and plants treated exogenously with methyl jasmonate (MeJA). Volatiles were collected continuously for 22 hr. Each bar represents the mean ± SE for five replicates. 1 = Hexanal; 2 = (*Z*)-3-hexenol; 3 = α-pinene; 4 = β-pinene; 5 = mycene; 6 = (*Z*)-3-hexenyl acetate; 7 = hexyl acetate; 8 = limonene; 9 = (*E*)-β-ocimene; 10 = linalool; 11 = (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 12 = methyl salicylate; 13 = indole; 14 = (*E*)-β-caryophyllene; 15 = α-humulene; 16 = (*E*)-β-farnesene; 17 = (*E*,*E*)-α-farnesene; 18 = tentatively identified as δ-cadinene [based on comparison of mass spectra with spectra published by Stenhagen et al. (1974) and the Eight Peak Index of Mass Spectra by the Mass Spectrometry Data Centre, Reading, UK]; 19 = nerolidol; 20 = (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

$P < 0.05$). This result was expected, since these compounds, commonly referred to as constitutive, are predominantly stored in the glands of cotton leaves (Elzen et al., 1985; McAuslane and Alborn, 1998).

Hexanal, (*Z*)-3-hexenol, and hexyl acetate, all LOX-pathway compounds, were detected in response to herbivore damage, but not in response to MeJA treatment (Figure 1). These compounds result from the breakdown of stored lipids (Hatanaka et al., 1987) and are released during insect damage (McCall et al., 1994), but not systemically (Röse et al., 1996). (*Z*)-3-Hexenyl acetate was the only LOX pathway volatile detected from MeJA treatment (Figure 1).

Timing of Induction. The emission of inducible terpenes followed a diurnal cycle for herbivore-damaged, MeJA-treated, and control plants (Figures 2–5). In general, volatile emission was much higher for plants treated with herbivores and MeJA compared to controls.Volatile emissions were highest during the period of highest radiation (from 10:00 to 14:00 hr and from 14:00 to 18:00 hr), and emissions decreased markedly during the night. Periods of maximum volatile emission corresponded to times of the day when leaf temperatures were the highest

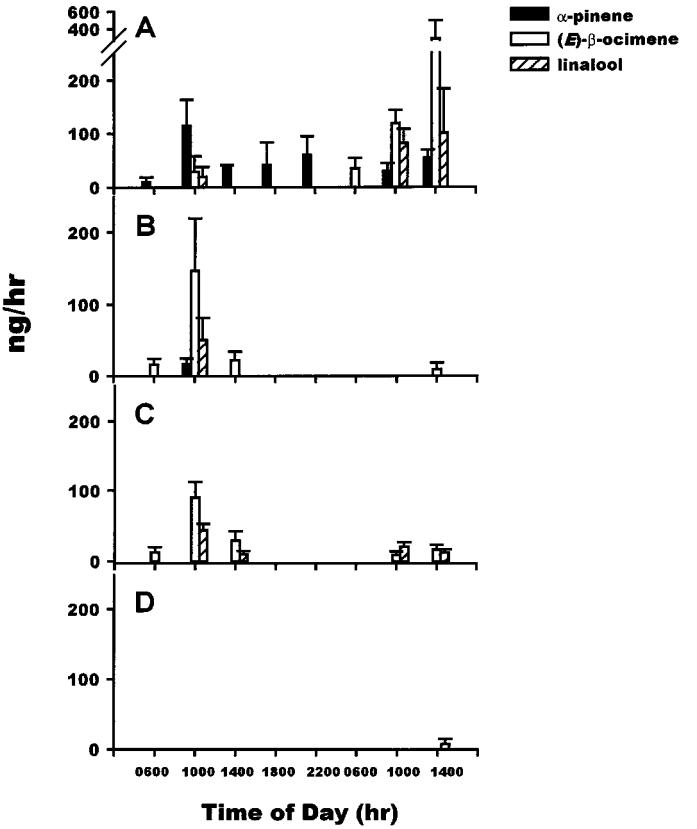

FIG. 2. Emissions of the monoterpenes α-pinene, (E)-β-ocimene, and linalool by aerial portions of cotton plants under continuous herbivore damage (A); with caterpillars removed from plants before being placed in collection chambers (B); treated overnight with exogenous methyl jasmonate prior to volatile collection (C); and undamaged controls (D). Each bar represents the mean $\pm$ SE for four replicates. Time of day indicates the time of initiation of each collection period and each collection represents a 4-hr interval.

[29.5 ± 0.3 (SE) and $35.5 \pm 0.5^{\circ}$C at 10:00 and 14:00 hr, respectively]. Lowest leaf temperatures were recorded at 06:00 hr ($22.9 \pm 0.4^{\circ}$C) and 22:00 hr ($22.6 \pm 0.5^{\circ}$C), which corresponded to the times of minimal volatile emission (Figures 2–5).

For plants subjected to continuous herbivore feeding, the emission of volatiles was highest during the second day of treatment (Figures 2A–5A). In contrast, when insect feeding was interrupted prior to collecting samples, volatile emission was much reduced on the second compared to the first day of the treatment (Figures 2B–5B). Additionally, plants treated with MeJA one time at the beginning of

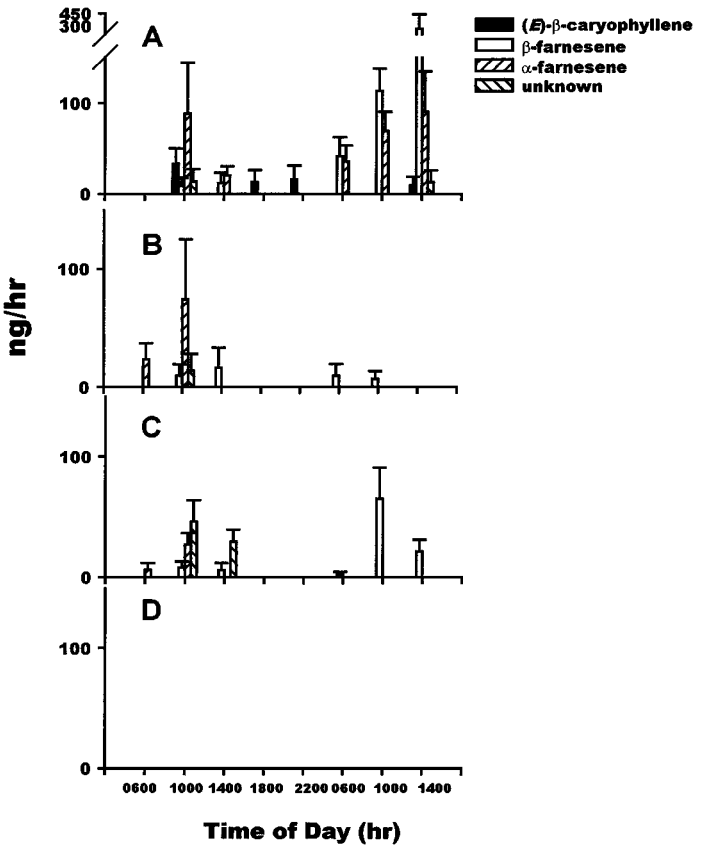

FIG. 3. Emissions of the sesquiterpenes (*E*)-β-caryophyllene, β-farnesene, α-farnesene, and an unknown by aerial portions of cotton plants under continuous herbivore damage (A); with caterpillars removed from plants before being placed in collection chambers (B); treated overnight with exogenous methyl jasmonate prior to volatile collection (C); and undamaged controls (D). Each bar represents the mean $\pm$ SE for four replicates. Time of day indicates the time of initiation of each collection period and each collection represents 4-hr interval.

the experiment had lower total volatile emission during the second, compared to the first, day of the experiment (Figures 2C–5C). In contrast to the inducible terpenes, the emission of the constitutive α-pinene and (*E*)-β-caryophyllene, released only from herbivore-damaged plants, did not follow a diurnal pattern (Figures 2A and 3A).

Systemic Induction. Both herbivore feeding and treatment with MeJA led to the systemic induction of volatile emission (Table 1; Figure 6). The blend of

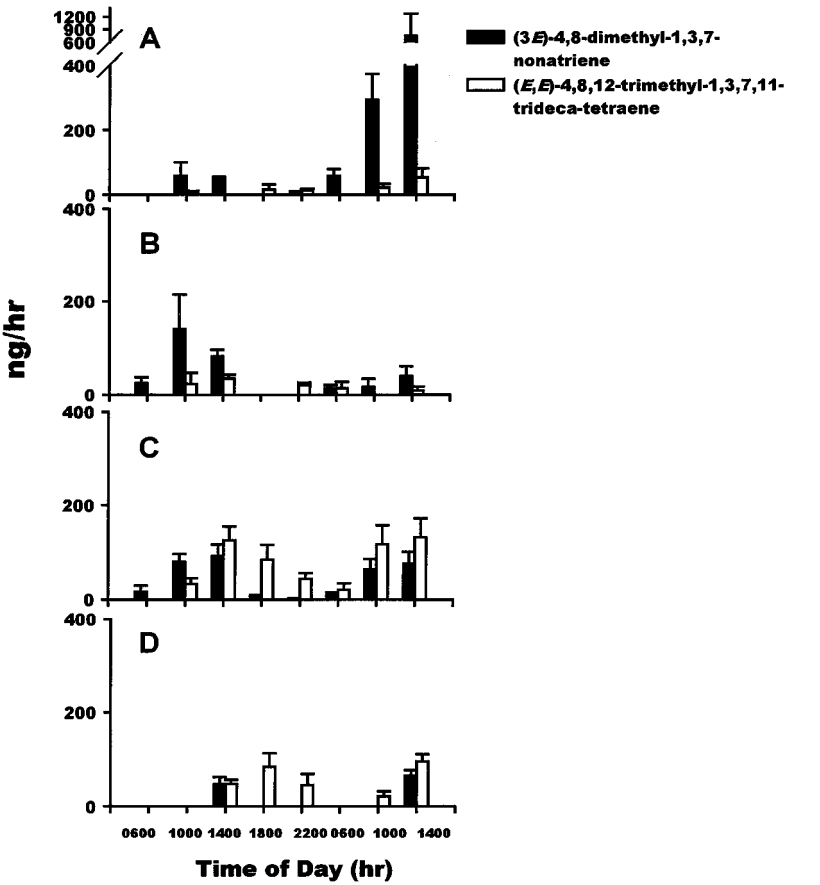

FIG. 4. Emissions of the homoterpenes (3*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene by aerial portions of cotton plants under continuous herbivore damage (A); with caterpillars removed from plants before being placed in collection chambers (B); treated overnight with exogenous methyl jasmonate prior to volatile collection (C); and undamaged controls (D). Each bar represents the mean ± SE for four replicates. Time of day indicates the time of initiation of each collection period and each collection represents a 4-hr interval.

volatiles emitted included (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-farnesene, (*E*,*E*)-α-farnesene, and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Figure 6). This systemically induced volatile blend was similar to the blend reported by Röse et al. (1996) for herbivore-damaged cotton.

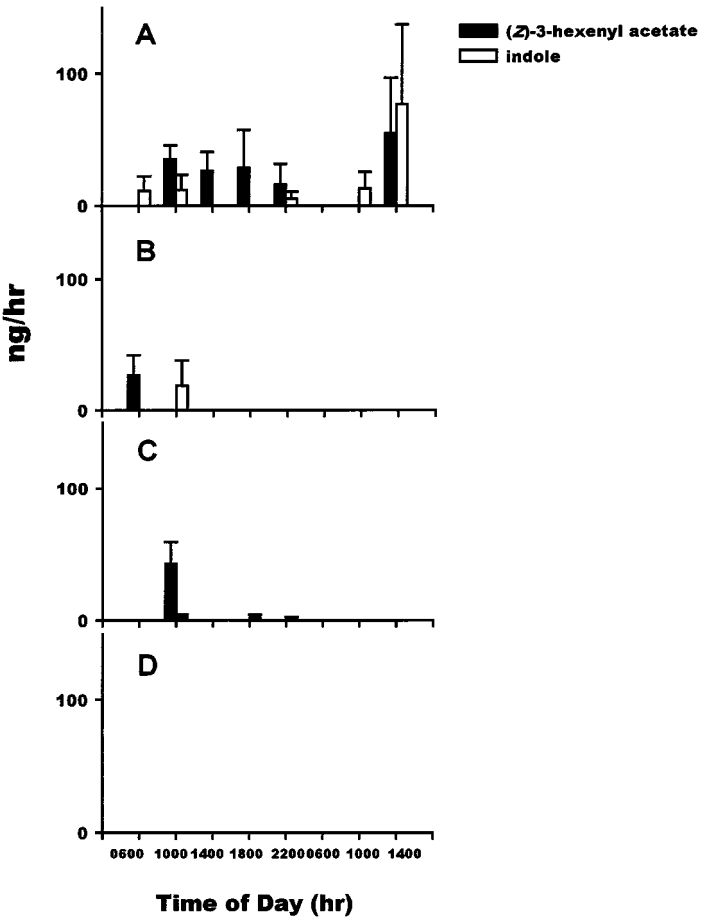

FIG. 5. Emissions of the LOX product (*Z*)-3-hexenyl acetate and indole by aerial portions of cotton plants under continuous herbivore damage (A); with caterpillars removed from plants before being placed in collection chambers (B); treated overnight with exogenous methyl jasmonate prior to volatile collection (C); and undamaged controls (D). Each bar represents the mean ± SE for four replicates. Time of day indicates the time of initiation of each collection period and each collection represents a 4-hr interval.

DISCUSSION

The induction of volatiles in cotton plants by herbivore damage has been documented (McCall et al., 1994), although the association of jasmonates in the induction of indirect defenses in cotton has not been previously reported. In cotton, MeJA mimicked the response of volatile emission induced by insects (Figure 1).

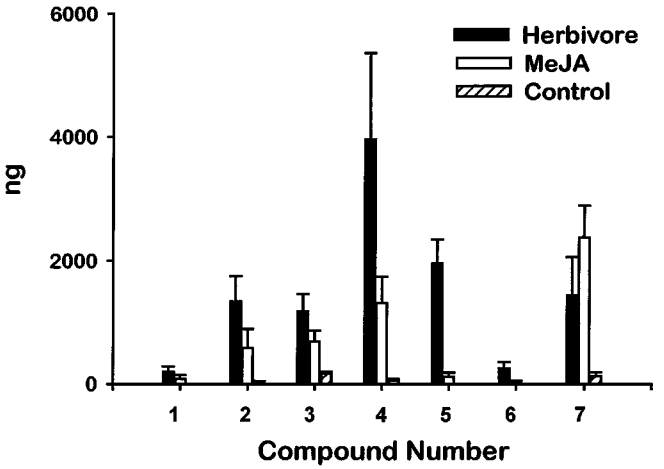

FIG. 6. Volatiles collected from the aerial portions of undamaged cotton plants (Control), undamaged leaves of cotton plants induced systemically by herbivore damage, and untreated leaves induced systemically by exogenous methyl jasmonate (MeJA). Cumulative volatiles emitted during the photoperiod of 4 consecutive days were collected. Each bar represents the mean ± SE for four replicates. 1 = (*Z*)-3-hexenyl acetate; 2 = (*E*)-β-ocimene; 3 = linalool; 4 = (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 5 = (*E*)-β-farnesene; 6 = (*E*,*E*)-α-farnesene; 7 = (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

In fact, a blend of volatiles released by cotton plants treated exogenously with MeJA contained all compounds synthesized *de novo* following herbivore damage (Paré and Tumlinson, 1997b). Methyl jasmonate did not induce emissions of stored terpenes, a result that was expected since the release of stored terpenes is dependent on physical damage caused by herbivores.

These results also indicate that MeJA activates multiple biosynthetic pathways involved in the synthesis of cotton volatiles: the classic mevalonic acid pathway leading to sesquiterpenes, the alternate isopentenyl pyrophosphate pathway producing the monoterpenes, the homoterpene pathway producing the C_{11} and C_{16} unit terpenes, the shikimic acid/tryptophan pathway leading to the synthesis of methyl salicylate and indole, and the LOX pathway that leads to (*Z*)-3-hexenyl acetate (Paré and Tumlinson, 1996, 1999).

Similar to reports by Loughrin et al. (1994) and Turlings et al. (1998), herbivore-induced volatile emission was highest during the photoperiod (Figures 2–5). Our results indicated that MeJA-induced volatile emission followed a diurnal trend similar to herbivore treatment (Figures 2–5). Thus, volatile emission is highest during the time when natural enemies tend to forage (Turlings et al., 1995), when leaf temperature is highest, and when leaves are conducting photosynthesis (Paré and Tumlinson, 1997a,b).

The induction of volatiles was greatly diminished over time when herbivore feeding was interrupted or when MeJA was applied only one time (Figures 2B,C–5B,C). This indicates that the induction is dependent on a continuous supply of a chemical signal. Obviously, the reduced emission of constitutive volatiles after insect feeding was stopped due to the cessation of physical damage by the insects (Figures 2B–5B).

Systemically induced volatile emission for herbivore-damaged plants has been reported for cotton and other plant species (Turlings and Tumlinson, 1992; Röse et al., 1996). Additionally, it has been shown that jasmonates induce volatile emission by plants (Boland et al., 1998; Dicke et al., 1999; Gols et al., 1999). Here we show for the first time that jasmonates can systemically induce the emission of volatile compounds. The systemic emission of volatiles points to a mobile signal or signals that move from damaged leaf to the undamaged portions of the plant. Paré and Tumlinson (1998) in studies with $^{13}CO_2$, have established that the systemically released volatiles are synthesized at the site of release. However, to date no systemic signals specific for volatile induction have been identified. Our results indicate that MeJA, or an induced product, was transported to distal leaves leading to the systemic emission of the same cotton volatiles systemically induced by *S. exigua* (Figure 6).

In conclusion, our results indicate that indirect defenses in cotton can be activated by MeJA. Volatiles released from cotton after herbivore feeding and induced by MeJA treatment provide cues for natural enemies to locate hosts (McCall et al., 1993; De Moraes et al., 1998). Our results suggest that jasmonates may be used as an elicitor of volatiles to attract natural enemies in cotton cultivars. For instance, in other plant systems, indirect evidence has been presented that volatiles induced by jasmonates serve as important cues for natural enemies to locate potential host/prey sites. Although volatiles were not measured, the aggregation of beneficial parasitic wasps was greater in field plots of tomato treated exogenously with JA than for untreated control plots (Thaler, 1999).

Recently, Dicke et al. (1999) showed that Lima beans treated with JA or MeJA produce a volatile blend that is similar to plants infested with the two-spotted spider mite *Tetranychus urticae* Koch. Jasmonate-treated plants attracted more predatory mites, *Phytoseiulus persimilis* Athias-Henriot, than untreated plants. Comparable results were obtained when gerbera leaves were treated with exogenous JA (Gols et al., 1999). Thus, it appears that jasmonates are potential agents that may be used to improve biological control in agricultural crops.

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